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***Nature Ecology and Evolution* 2017, 1, 0051.**

Bioaccumulation of persistent organic pollutants in the deepest ocean fauna

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The legacy and reach of anthropogenic influence is most clearly evidenced by its impact on the most remote and inaccessible habitats on Earth. Here we identify extraordinary levels of persistent organic pollutants in the endemic amphipod fauna from two of the deepest ocean trenches (>10,000 metres). Contaminant levels were considerably higher than documented for nearby regions of heavy industrialisation, indicating bioaccumulation of anthropogenic contamination and inferring that these pollutants are pervasive across the World's oceans and to full ocean depth.

The oceans comprise the largest biome on the planet with the deep ocean operating as a potential sink for the pollutants and litter that are discarded into the seas[1]. The spatial and bathymetric expanse of the deep sea infers that there are still large areas untouched by anthropogenic activity, although the intrinsic linkages between the deep sea and surface waters[2] would suggest this inference is ill-conceived[3]. The hadal zone (6000 to 11,000 m deep) is comprised of trenches formed at tectonic subduction zones and represents the least explored ecosystem on Earth and the last major marine ecological frontier[4]. Trenches have been considered both as pristine environments, but also given their locations and topography as likely sinks for contaminants that enter the marine environment[2]. Of particular concern are the persistent organic pollutants (POPs) that are highly detrimental to organismal health through their endocrine disrupting properties[5]. POPs possess an inherent hydrophobicity that confers a high binding affinity to organic or inorganic particles present in the water column that through vertical transport will collect in the deep ocean. They also have inherent lipophilicity, meaning these compounds readily bioaccumulate in organisms, with cumulative increases at each trophic level[6].

POPs were released into the environment through industrial accidents and discharges, leakage from landfills or incomplete incineration[7]. Two key POPs are polychlorinated biphenyls (PCBs, used as dielectric fluid) and polybrominated diphenyl ethers (PBDEs, used as flame retardants). From the 1930s to when PCB production ceased in the 1970s, the total global production was ~1.3 million tonnes[8]. Approximately 65% is thought to be contained in landfills or still within electrical equipment, with the other 35% residing in coastal sediments and open oceans[9]. These pollutants are invulnerable to natural degradation[10] and so persist in the environment for decades. Moreover they can spread great distances, including to seemingly isolated environments, such as polar regions and open ocean[3].

Pollutants entering the deep sea are deposited in sediments and can readily accumulate into the food-chain[11]. Studies on deep-sea organisms have reported higher concentrations than in nearby surface water species[11,12]. However, although these studies are described as 'deep sea' they rarely extend beyond the continental shelf (<2000m), so contamination at greater distances from shore and the extreme depths is hitherto unknown.

We measured the concentrations of key PCBs and PBDEs in multiple endemic and ecologically equivalent Lysianassoid amphipod Crustacea from across two of the deepest hadal trenches - the oligotrophic Mariana Trench in the North Pacific, and the more eutrophic Kermadec in the South Pacific. Two endemic amphipods (*Hirondellea dubia* and *Bathycallisoma schellenbergi*) were sampled from the Kermadec between 7227 and 10,000 m, and one (*Hirondellea gigas*) from the Mariana between 7841 and 10,250 m. Samples were obtained using traps deployed on deep-sea landers[13]. The concentrations of seven PCB congeners identified by ICES[14] for marine pollution assessment and seven PBDE congeners were measured both in sample dry weight (dw) and lipid weight (lw).

The salient finding was that PCBs and PBDEs were present in all samples across all species at all depths in both trenches. The ΣPCB7 concentrations ranged from 147.3-905 ng g^{-1} dw in the Mariana and 18.03-42.85 ng g^{-1} dw in the Kermadec, with mean values of 382.28 ng g^{-1} dw ±281.6 S.D and 25.24 ng g^{-1} dw ± 9.1 S.D respectively Across individual PCB congeners, PCB 153 was detected in the highest concentration (mean 64.45; range 5.03–373.63 ng g^{-1} dw). Congeners PCB 138 and 153 alone accounted for 65% of the total PCB concentration, which suggested the heavier congeners were more recalcitrant towards degradation.

ΣPBDE7 concentrations ranged from 5.82-28.93 ng g^{-1} dw in the Mariana Trench and 13.75-31.02 ng g^{-1} dw in the Kermadec. Concentrations of congeners PBDE 153 and 154 were below limit of detection (LOD) in some samples, while PBDE 183 was not detected. Congener PBDE 47 and 99 were found the higher concentrations (mean: 8.81, range 2.55–21.36 ng g^{-1} dw; mean 3.31, range 0.78-8.38 ng g^{-1} dw respectively) and accounted for 71% of the total PBDE concentration.

For both ΣPCB7 and ΣPBDE7 there were significant correlations between dry and lipid weights (Pearson's $r = 0.98$ $p = 8 \times 10^{-9}$ and $r = 0.70$, $p = 0.012$, respectively). There was no statistically significant relationship between concentration and depth within either trench (see supplementary information). In both trenches the highest values were found in the upper trench; 7841 m in the Mariana and 7227 m in the Kermadec.

These data clearly indicate that potent anthropogenic contamination and bioaccumulation has occurred in a dominant macrofaunal group inhabiting the complete depth range of two of the deepest marine trenches. Placing the levels of contamination into a broader comparative context with values published from the western Pacific is complicated by variations in POP congeners assayed across different studies and level of replication. Notwithstanding, some insightful comparisons are possible. World baseline levels for ΣPCBs arising from atmospheric transport found in clean coastal sediments are <1 ng g^{-1} dw[15]. In grossly polluted areas, levels can be far higher[16], reaching up to 314 ng g^{-1} dw in in Guam, 240 ng g^{-1} dw in Japan and 160 ng g^{-1} dw in Australia. Indeed, in the Mariana, the highest level of PCBs were fifty times more contaminated than crabs from paddy fields fed by the Liaohe River, one of the most polluted rivers in China[18]. The only NW Pacific location with values comparable to the Mariana Trench is Suruga Bay (Japan), a highly industrialised area with historically heavy usage of organochlorine chemicals[19].

Contamination by PBDEs did not reach the magnitude of PCB levels. The closest data for PBDE levels to the Kermadec Trench are estuarine sediments from New Zealand's North Island (0.55 to 573 ng g^{-1} dw)[23]. These results are however highly variable with some locations, such as Puketutu Island, being exceptionally high (>500 ng g^{-1} dw). However, the median value of 10.3 ng g^{-1} dw is considerably lower than the Kermadec Trench (18.4 ng g^{-1} dw) and close to that in the Mariana Trench (10.44 ng g^{-1} dw). The levels of PBDEs from New Zealand estuaries were not considered excessively high[23]. Notwithstanding, the salient finding is that PBDEs are present in the hadal samples at comparable or higher levels than in coastal waters.

PCB concentrations were notably higher in the Mariana than in the Kermadec Trench. There are several possible explanations that are not mutually exclusive and at this juncture speculative. First, the high levels of the Mariana PCBs may originate from proximity to the industrialised regions in the NW Pacific[21] and the North Pacific Subtropical Gyre, famed for its reputation as the 'Great Pacific Garbage Patch'[22]. As such, it is located beneath a mass accumulation of trapped plastic debris which ultimately sinks as the plastics degrade and fragment, transporting POPs to depth. Second, the amphipods may be accumulating POPs through surface derived carrion-falls that have experienced contrasting levels of contamination in the surface. This however appears unlikely given the consistent patterns of contamination at different depths and the oligotrophic nature of the Pacific around the

Mariana. Third, it may also be likely that the different species bioaccumulate these contaminants differently through physiological differences, lipid turnover rates, metabolic capacity, and ingestion throughput. Again, this is somewhat less likely given the ecological equivalence of the species though there is a lack of information on their respective physiologies.

The most parsimonious explanation for PCB and PBDE accumulate in these remote and deep trenches is via long range oceanic and atmospheric transport and association with particulate matter and carrion-falls sinking through the water column. The potentially rapid rate of vertical transportation of surface derived material was clearly demonstrated following the 2011 Fukushima Dia-ichi nuclear disaster where the detection of ^{134}Cs radiation at 7553 m deep in the Japan Trench suggested a sinking rate of 64 to 78 m day^{-1} [24]. Therefore the travel time between the surface and seafloor at full ocean depth is between ~110 and 170 days, and likely shorter for carrion-falls. Given that 370 x 10^3 t of PCBs are estimated to now reside in the oceans[9], a rapid influx of surface material suggests little bathymetric variation of seafloor concentrations. Indeed, topographically closed trench morphologies may hamper contaminant dispersal.

The extreme hydrostatic pressures that characterise the hadal zone require non-trivial evolutionary adaptations for survival, and present major engineering challenges in accessing full ocean depth. Such statements underpin the perspective that the hadal zone is remote and inaccessible, with popularist analogies generally reinforcing this view, such that if Mount Everest was placed into the Mariana Trench its summit remains a mile below the surface. However, the distance from the surface to full ocean depth is actually only equal to the widest point of the Mississippi River, and half the length of Manhattan Island. These alternative views emphasize that our proximity to these extreme locations is far from remote, which is why even the deepest chasms of the ocean are no longer pristine. The challenge moving forward is to determine the physiological consequences of such contamination and understand knock-on effects on ecosystem function.

Acknowledgements. The Kermadec and Mariana 'HADES' expeditions (RV *Thomas G. Thompson* TN309, and RV *Falkor* FK141109) were funded through the National Science Foundation (NSF-OCE #1130712, 1140494) and the Schmidt Ocean Institute. SBP was supported by a Fellowship from the Leverhulme Trust. The analytical costs were supported by the Total Foundation (France) and the Marine Alliance for Science and Technology, Scotland (MASTS) through a Deep Sea Forum small grant award.

Author contributions. A.J.J. conceived the experiment, designed the sampling equipment and was awarded the analytical costs. A.J.J. and S.B.P. performed the sampling at sea. S.B.P identified species,

and T.M. performed the laboratory analysis under the supervision of Z.Z. and A.J.J. T.M. and T.F. performed the statistical analyses and the manuscript was written by A.J.J., T.M., S.B.P. and Z.Z.

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Data availability. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Figures

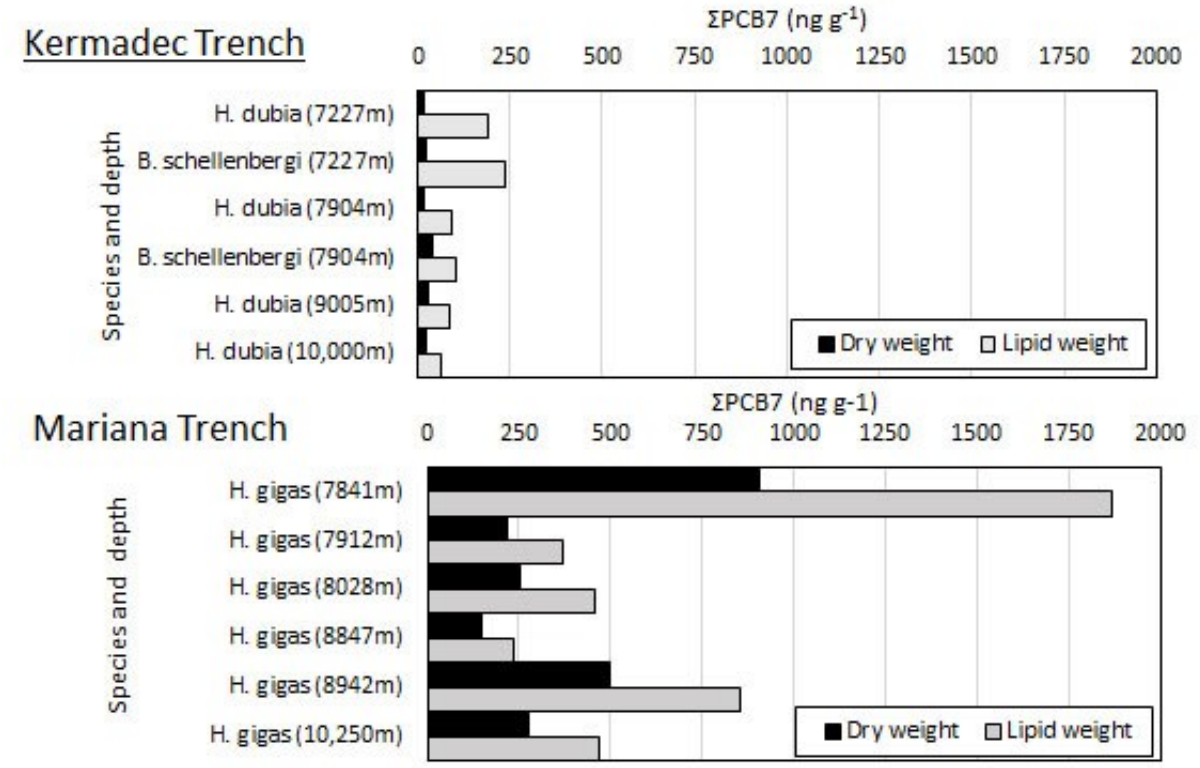

Figure 1. Polychlorinated biphenyl (PCB) concentration (ng g^{-1}) for both dry and lipid weight found in endemic hadal amphipods across the bathymetric ranges of the Kermadec and Mariana trenches.

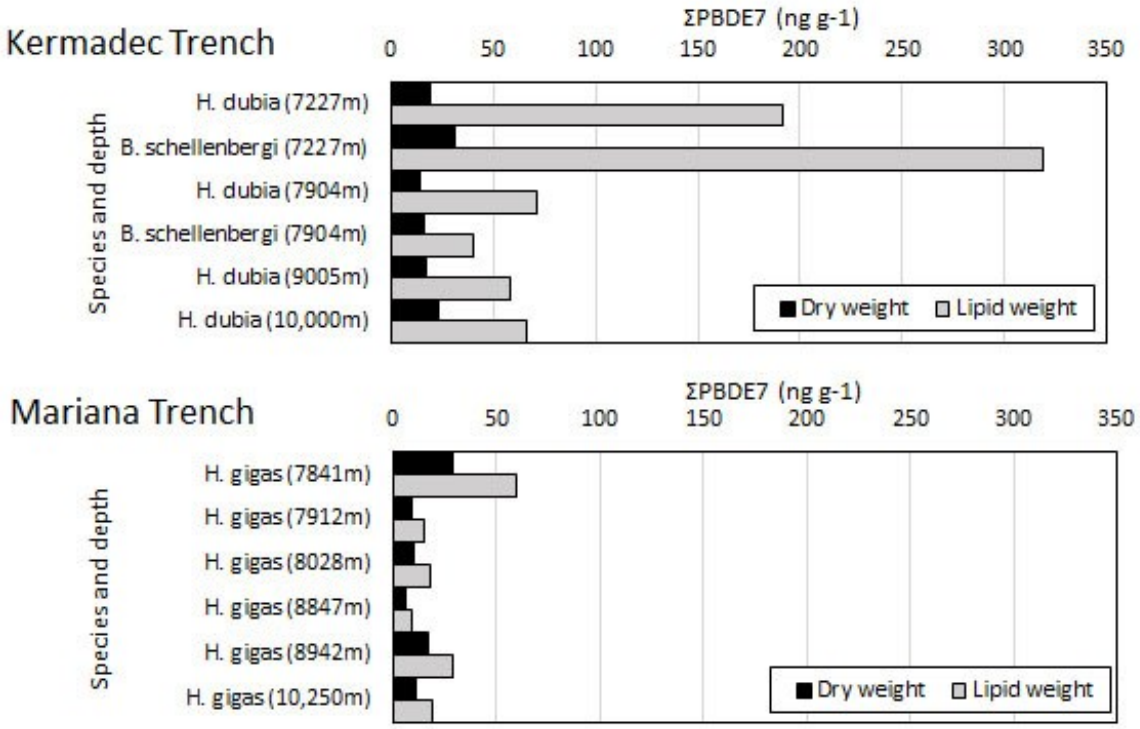

Figure 2. Polybrominated diphenyl ether (PBDE) concentration (ng g^{-1}) for both dry and lipid weight found in endemic hadal amphipods across the bathymetric ranges of the Kermadec and Mariana trenches.

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METHODS

Sampling - Samples were collected using a full ocean depth rated lander vehicle[25] deployed to the seafloor by free-fall and acoustically recalled by jettisoning ballast weights. The lander typically remained on the seafloor for 8 to 12 h. Coupled to the lander footpads were a cluster of three small funnel traps (30 cm length by 6 cm diameter with trap openings of approximately 2.5 cm diameter). The traps were baited with ~100 g of mackerel that was enclosed in a mesh bag to allow the development of an odour plume, but prevent the amphipods consuming any of the bait that might otherwise affect POP levels in downstream assays. Once the samples were landed on deck they were not exposed to any plastics during preparation or preservation. The samples were transferred to a cold room (4 °C) on glass plates and sorted using metal implements before being transferred into pre-muffled (450 °C) foil wrapped tightly in several layers and frozen at -80 °C in natural fibre pouches.

Study sites and species – Samples of Lysianassoid amphipods were obtained from across two of the deepest hadal trenches - the Mariana Trench that underlies the oligotrophic surface waters of the North Pacific, and the Kermadec Trench situated under moderate primary productivity off New Zealand in the South Pacific. Two endemic species of amphipod (*Hirondellea dubia* and *Bathycallisoma schellenbergi*) were sampled from the Kermadec (32° S 177° W), at depths of 7227, 7904, 9005 and 10,000 m. One species of endemic amphipod (*Hirondellea gigas*) was sampled from the Mariana (12° N 145° E), at depths of 7841, 7912, 8028, 8847, 8942 and 10,250 m. In all cases, samples were obtained using traps deployed on autonomous deep-sea lander vehicles[25].

Contaminant analyses. The concentrations of seven PCB congeners (28, 52, 101, 118, 138, 153 and 180) identified by ICES[14] for marine pollution assessment and seven PBDE congeners (28, 47, 99, 100, 153, 154 and 183) with a wide range of bromination. Concentrations of PCBs and PBDEs in amphipods were determined using gas chromatography/mass spectrometry (GCMS), following extraction and sample clean-up, using method described previously[26]. Procedural blanks were run in parallel with each batch of samples and the results were corrected accordingly. The mean recoveries of the method were 40-77% for PCBs and 71-93% for PBDEs, respectively. Limits of detection (LOD) were 0.02 ng g^{-1} for all PCBs and PBDE congeners 28, 47, 99 and 100, and 0.50 ng g^{-1} for PBDE congeners 153, 154 and 183.

Lipid content. Total lipid content was assessed using a single step extraction method[27]. 90 mg of sample were extracted with 2:1 chloroform-methanol (v/v) mixture in a glass centrifuge tube. After adding NaCl water solution, the phase separation was facilitated by centrifugation to recover the chloroform phase into a clean, weighed scintillation vials. Chloroform was evaporated and the vials were re-weighed to calculate total lipid content (%) for each sample.

Statistical Methods. Simple linear regression was performed to examine the possible effect of depth on the measurements of each pollutant (i.e. PCB7 dw, PCB7 lw, PBDE7 dw and PBDE7 lw) in both trenches (supplementary information Table 3). The pollutant measurements were highly skewed and therefore natural-log transformation was applied to normalise and stabilise the variance. Estimated regression model residuals were examined for statistical adequacy using standard graphical tools to verify the assumption of normality and homogeneity. In addition, Welch's t-test was performed to test for the difference in the respective pollutant levels between the two trenches (supplementary information Table 4). For the results of both linear regression analysis and Welch's t-test, adjusted significance levels ($\alpha = 0.05$ divided by n=8 and 4, respectively) were applied using the Bonferroni inequality correction. All analyses were performed using R v3.2.3 package (R Development Core Team, 2015).

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